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# Neoangiogenesis after combined transplantation of skeletal myoblasts and angiopoietic progenitors leads to increased cell engraftment and lower apoptosis rates in ischemic heart failure☆

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**Abstract**

**Objectives:** We previously reported that combined transplantation of skeletal myoblasts and AC-133+ cells leads to improved left ventricular function, reduced infarct size and myocardial apoptosis in a model of chronic ischemia. The aim of this study is to elucidate on the possible mechanisms and to assess new implications in increasing cell therapy efficacy in chronic ischemia. **Methods:** Heart failure was induced by LAD-ligation in nude rats. (a) Homologous skeletal myoblasts (SM), (b) human derived AC-133+ cells (SC), (c) combination of both cells (Comb) and (d) culture medium (CM) were injected in the infarct and peri-infarct area, respectively, four weeks after infarction. Cell engraftment was detected by fluorescence microscopy and confirmed by immunohistochemical techniques. Cardiac gene expression levels of VEFG-A, cardiac troponin, ACTA2, SDF-1, TGF-beta-1, were assessed by RT-PCR. **Results:** Both cell types were detected in the injection areas four weeks after cell transplantation. Double cell therapy led to increased cell engraftment (SM: $52\pm13/mm^2$, SC: $45\pm8$ in the combination group vs. SM: $31\pm9$ and $23\pm7$ in the monotherapy groups, $P=0.007$). This effect was confirmed using PCR. Apoptotic index among engrafted cells was significantly lower in the Comb group (Comb: $0.53\pm0.12$ for myoblasts and $0.34\pm0.09$ for SC, vs. SM: $0.76\pm0.19$ and SC: $0.63\pm0.16$, $P=0.013$). Expression of cardiac troponin was higher in the combination group in the peri-infarct area. Evaluation of capillary density revealed increased angiogenesis in the combination group (Comb: $12.3\pm2.3$, SM: $5.2\pm1.2$, SC: $8.3\pm1.8$, $P=0.002$). Neoangiogenesis was associated with higher levels of VEGF-A and TGF-beta in the injection areas as detected by RT-PCR. The higher SDF-1 expression in the injected areas implies an increased secretion of chemoattractants by the injected cells, which suggests that the effect of combined cell transplantation is mainly associated with paracrine mechanisms. **Conclusions:** The mechanism of functional improvement after combined transplantation of skeletal myoblasts and AC-133+ progenitors in ischemic heart failure is mainly associated with increased angiogenesis based on paracrine factors, which leads to improved survival and lower apoptosis rates of the injected cells.


*Keywords:* Myocardial infarction; Ischemic heart failure; Skeletal myoblasts; Stem cell therapy; Angiogenesis

## 1. Introduction

Gene therapy was based on the assumption that local delivery of several chemoattractive proteins in the cardiac muscle may stimulate angiogenesis and the mechanisms of intrinsic repair in infarcted myocardium. This can be performed either by direct transfer of genes into the patient or by using living cells as vehicles to transport the genes of interest. The rationale of cell-based gene therapy is to use living cells as vehicle for gene transfer, and this combination may have a synergistic beneficial effect on the failing heart [1].

Although this strategy has been proposed during the first attempts of cell therapy, replacement of gene delivery by a second cell type seems to have several advantages: no viral vector is anymore needed, so that many of its technical shortcomings and side effects can be avoided, overexpression of several genes can be achieved by using a specific cell type.

Combined cell therapy for cardiac regeneration is founded on two principal assumptions: first, that it is possible to improve efficacy by merging the beneficial effects of each cell line, and second, that cell dose can be reduced in order to avoid side effects and facilitate technical issues [2]. The first attempts included one from our group and demonstrated improved efficacy of combined transplantation of skeletal myoblasts (SM) and bone marrow-derived mononuclear stem cells (BM-MNC) in acute and subacute myocardial infarction. In combination with BM-MNC, half of the number of transplanted SM was as effective in restoring left ventricular function and inducing angiogenesis as in single cell transplantation [3, 4].

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In a recent study, our group evaluated the effect of combined transplantation of SMs and AC-133+ angiopoietic progenitor cells in a rodent model of ischemic heart failure [5]. Therefore, we selected a specific subgroup of endothelial progenitor cells, which represent only about 1% of the cells in whole bone marrow aspirates or whole blood from G-CSF-mobilized patients, and which have been shown to actively participate in new vessel formation [6]. The combined transplantation of these cell types resulted in improved ventricular function and attenuated left ventricular dilatation, which correlated to histologic findings of reduced myocardial fibrosis and apoptotic rates in the peri-infarct region and the distal area. Moreover, neovascularization was significantly higher in the combination group compared to controls and single cell therapy groups.

Although both cell types seem to play different roles in cardiac regeneration and neoangiogenesis, the presence of cellular interactions at molecular level might be responsible for the enhanced efficacy of combination therapy. However, the exact mechanism of action and the extent to which both cell types communicate with each other remains to be investigated. The aim of this study was to delve into the mechanisms of the functional and morphological improvement after injecting skeletal myoblasts in the infarct scar and AC-133+ angiopoietic progenitors in the peri-infarct area in order to shed light to this new cell therapeutic strategy.

## 2. Methods

### 2.1. Model of myocardial infarction and ischemic heart failure

Myocardial infarction was induced in 8–10 weeks old male athymic nude rats (rnu/rnu Harlan Winkelmann, Borchen, Germany) through LAD-ligation. All animals received human care in compliance with the 'Principles of laboratory animal care' formulated by the National Society for Medical Research and the 'Guide for the care and use of laboratory animals', prepared by the Institute of Laboratory Animal Resource and published by the NIH.

### 2.2. Isolation, labeling and culture of skeletal myoblasts from syngeneic rats

Rat skeletal myoblasts were isolated from 6 weeks old male F344 rats (syngeneic rats). Isolation, culture and expansion procedures have been described previously [14]. Stable transgenic rat skeletal myoblasts were generated by a plasmid encoding for the yellow fluorescence protein (YFP, Invitrogen Corporation, Austria) containing the neomycin resistance gene for subsequent selection of stable YFP+clones. Purity of clonal myoblast cultures was proved by means of desmin immunohistology (IMMH-5, Sigma) and anti-skeletal myosin fast antibodies (clone My-32, Sigma). All sections were overlaid to pictures obtained by fluorescence microscopy and were double confirmed using myosin heavy chain fast-slow staining (clone My-32, Sigma) to confirm cell-tracking. Long-term cell survival was quantified by counting My-32+cells per mm$^2$.

### 2.3. Isolation, purification and labeling of human-derived AC-133+ angiopoietic progenitors

Mononuclear cells were obtained from single-donor leukapheresis products of individuals mobilized with recombinant G-CSF 10 μg/kg s.c. (Neupogen, Amgen) for at least four days. Highly-purified CD133+ cells (>98% positive) were obtained within 48 h after blood collection using magnetic beads coated with monoclonal antibodies against CD133 (Miltenyi Biotech, Bergisch-Gladbach, Germany). Cell purity was controlled by means of flow cytometry using antibodies against CD45 and CD133 (Becton Dickinson, Franklin Lakes, NJ). AC-133+ cells were cultured with 10 μg/ml lipoprotein labeled with carbocynine membrane 1,1-dioctadecyl-3,3,3,3-tetramethyl indocarbocyanine perchlorate (Cell-Tracker CM-DiI, Molecular Probes, Leiden, The Netherlands) for subsequent cell tracking. After cell viability assessment (propidium iodide staining), aliquots of $10^6$ AC-133+ cells in approximately 200 μl culture medium (RPMI) were prepared for intramyocardial injections. Cell tracking included DiI fluorescence detection using fluorescence microscopy and counter-staining with human-specific anti-HLA Class I (clone w6/32, Sigma) using immunochistochemistry. Long-term cell survival was quantified by counting human-specific anti-HLA Class I+cells per mm$^2$.

### 2.4. Cell transplantation

Four weeks after myocardial infarction and after echocardiographic verification of chronic heart failure, rats were reanesthetized and hearts were reexposed through left redo-thoracotomy in order to allow access to the infarct area and the peri-infarct rim proximally and distally to the scar. RPMI-culture medium ($n=10$), $10^6$ YFP-labeled, desmin-positive skeletal myoblasts from syngeneic rats ($n=12$), $10^6$ DiI-labeled AC-133+ human-derived angiopoietic progenitor cells ($n=12$), and a combination of $5\times10^5$ YFP-labeled, desmin-positive skeletal myoblasts from syngeneic rats, and $5\times10^5$ DiI-labeled AC-133+ human-derived angiopoietic progenitor cells were injected in the peri-infarct area and in the scar ($n=12$). Skeletal myoblasts and AC-133+ cells have been exclusively injected in the infarct scar and the peri-infarct area, respectively.

### 2.5. Real-time PCR (rt-PCR)

Expression of various genes was examined by rt-PCR. Samples were taken under sterile conditions, the ischemic areas were excised and placed in RNAse free tubes and stored at −80 °C. Poly(A)$^+$ mRNA was extracted using TRI-Reagent (MBI, Minneapolis, MN) from the infarct area or the rest myocardium from all animal groups. RNA extracted from $1\times10^6$ cells was primed and reversed transcribed using Moloney murine leukemia virus reverse transcriptase (Promega, Madison, WI). Thirty circles of PCR were done using Taq polymerase (Promega) and specific forward and reverse primer pairs for human and ratVEGF-A (vascular endothelial growth factor A), human and ratMYH6 (myosin heavy chain), human and ratTNNT2 (cardial troponin T2), human and ratSDF-1 (stromal cell-derived factor 1), human and rat TGF-beta-1 (transforming growth factor beta-1),

ACTA 2 (smooth muscle alpha actin 2). Reverse transcription of human and rat TBP (TATA-Box binding protein) was used as an invariant endogenous control. PCR products were visualized under UV light after gel electrophoresis in 2% agarose containing ethidium bromide. Results were expressed as number of DNA molecules per one million of 18S-RNA in both infarct and non-infarct areas.

### 2.6. Animal sacrification and preparation for morphological studies

Rats were euthanatized, hearts were harvested, fibrous tissues were removed and after rinsing intracardiac blood, they were divided into three equally thick parts representing the base, the middle and the apex of the heart. Each of them was snap frozen in liquid nitrogen after being embedded in OCT compound (Tissue-Tec OCT Compound, Miles Inc., Elkhart, IN). From each part, 5 μm slides were prepared using cryostat. Transplanted cells were detected by confocal fluorescence microscopy. Additionally, standard hematoxylin-eosin staining was performed to permit morphological assessment.

### 2.7. Evaluation of capillary density and expression of Connexin-43

To detect capillaries in the myocardium, frozen transverse sections were randomly obtained from all three heart parts and stained using an anti-CD34 monoclonal antibody (clone QBEnd-10). Immunohistochemical visualization was performed by using the EnVision™ kit (all Dako Cytomation, Glostrup, Denmark). The number of capillaries was counted in the border zone and the distal area. Capillary density was expressed as the average number of capillaries of five randomly high power fields (HPF). Additional Connexin-43 staining (anti-rat Connexin-43 antibody, Dako Cytomation, Glostrup, Denmark) was performed to detect integration of engrafted myotubes in the host myocardium.

### 2.8. Evaluation of apoptotic index in transplanted cells

For in situ detection of apoptosis at the single cell level transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL) staining was performed using the in situ cell death detection lit/peroxidase (POD) (Roche, Indianapolis, IN), according to the instructions of the manufacturer. Double staining of human-specific anti-HLA Class I (clone w6/32, Sigma) for detection of AC-133+ progenitors and TUNEL or rat anti-skeletal myosin fast antibodies (clone My-32, Sigma) and TUNEL were performed to detect apoptosis of engrafted cells. Within each field all cell engrafted areas both in the peri-infarct rim and the scar were examined. The results were expressed as apoptotic index representing the ratio of apoptotic cells at the total cell population. Both cell types injected were analyzed separately.

### 2.9. Data analysis

Statistical analysis was performed using SPSS 11.0 for Windows (SPSS, Chicago, IL). Data are expressed as mean ± S.D. Comparisons of continuous variables among animal groups were studied by one-way ANOVA. Longitudinal studies comparing data within each group were achieved by the use of paired *t*-tests. A value of $P<0.05$ after Bonferroni correction was considered significant.

## 3. Results

### 3.1. Engraftment of AC-133+ cells and skeletal myoblasts in the peri-infarct area and in the scar

Transplanted AC-133+ progenitors have been detected in the peri-infarct area four weeks after transplantation using conventional fluorescence microscopy (Fig. 1a). In order to avoid fluorescence detection of dead or apoptotic cells additional immunohistochemical staining against human-specific anti-HLA Class I antibody was performed (Fig. 1b). The survival rates of human-derived AC-133+ progenitors in the peri-infarct area was significantly higher in the double cell therapy group, as compared with the monotherapy group (Comb: 45 ± 8 cells/mm² vs. SC: 23 ± 7 cells/mm², $P=0.002$). Accordingly, we were able to detect transplanted skeletal myoblasts forming myotubes in the infarct area using fluorescence microscopy (Fig. 1c). As shown in Fig. 1d, this was additionally confirmed by means of immune histochemistry against rat-my-32, showing higher survival rates of the transplanted cells in the combination group (Comb: 52 ± 13 cells/mm², vs. SM: 31 ± 9 cells/mm², $P=0.007$). In order to evaluate integration of myotubes into the host myocardium, expression of rat-connexin-43 was analyzed by immune histochemistry. As shown in Fig. 2, connexin 43 is not expressed neither within engrafted skeletal myoblasts nor within myotubes and host myocardium, confirming that skeletal myoblasts represent isolated contractile islets in the scar, which are not functionally integrated to the host myocardium.

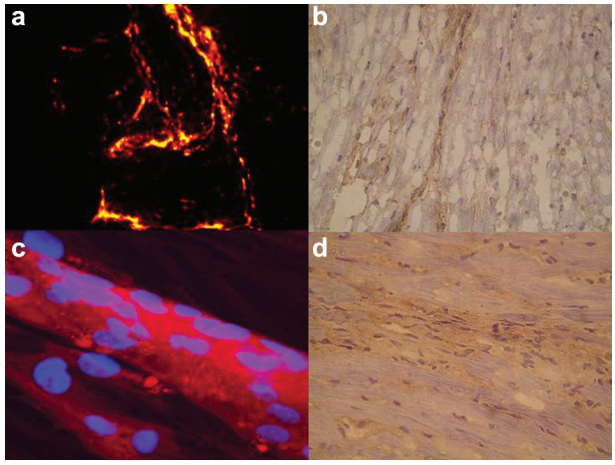


Fig. 1. (a) Detection of DiI-labeled human AC-133+ cells in the peri-infarct area using fluorescence microscopy and (b) confirmation by means of immunohistochemical staining against human-specific anti-HLA Class I. (c) Detection of rat skeletal myoblasts in the infarct scar using immunofluorescence against my-32, and (d) confirmation by means of immunohistochemical staining against my-32.

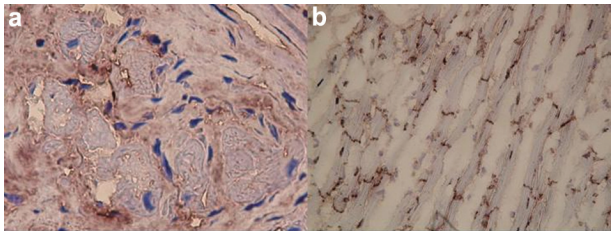


Fig. 2. Expression of connexin-43 (a) of engrafted skeletal myoblasts in the infarct scar after skeletal myoblast transplantation group and (b) after culture medium injections.

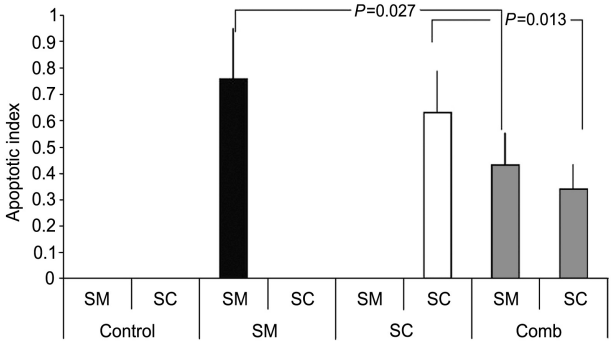


Fig. 3. Apoptotic index using TUNEL staining after stem cell- (open bars), skeletal myoblast- (closed bars), and combined stem cell and skeletal myoblast transplantation (gray bars), showing lower apoptosis rates of the transplanted cells after double cell therapy.

### 3.2. Apoptosis levels of engrafted cells are lower in the combination group

In order to assess functionality of the engrafted cells, we additionally performed apoptosis studies in both transplanted cell types. As shown in Fig. 3, lower apoptotic indices were detected after combined cell therapy within AC-133+ progenitors (Comb: $0.34 \pm 0.09$ vs. SC: $0.63 \pm 0.16$, $P = 0.013$). Moreover, TUNEL staining also revealed lower apoptosis rates of transplanted skeletal myoblasts in the combination group (Comb: $0.43 \pm 0.12$ vs. SM: $0.76 \pm 0.19$, $P = 0.027$).

### 3.3. Increased expression of my-32 confirms improved survival of transplanted skeletal myoblasts

Gene expression analysis of my-32 was used to confirm survival of the transplanted in the infarct scar. As shown in Fig. 4, RT-PCR analysis revealed a significantly higher expression of my-32 in the scar of animals after combined transplantation of skeletal myoblasts and AC-133+ progenitors as compared to animals which received skeletal myoblasts alone (Comb: $1907.8 \pm 134.6$ DNA molecules in one million 18S RNA vs. $1320.8 \pm 273.6$ DNA molecules in one million 18S RNA, $P = 0.025$). As skeletal myoblasts have been injected solely in the scar, my-32 expression in the peri-infarct area was similarly low in all groups.

### 3.4. Higher expression of Troponin T in the peri-infarct area

Troponin T levels were higher in the peri-infarct zone of the combination group as compared both to the control

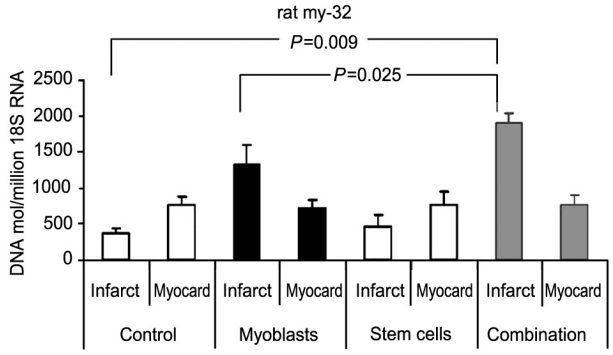


Fig. 4. Increased expression of myosin heavy chain (my-32) in the infarct scar after combined transplantation of skeletal myoblasts and AC-133+ progenitor cells (gray bars), as compared to isolated stem cell- (open bars), myoblast transplantation (closed bars) or controls (dotted bars) as detected by rt-PCR.

and the cell monotherapy groups (Comb: $18203 \pm 4852.4$ DNA molecules in one million 18S RNA, vs. SC: $17208.9 \pm 3927$ DNA molecules in one million 18S RNA, vs. SM: $13904.4 \pm 1040.3$ DNA molecules in one million 18S RNA, vs. Control: $2868.8 \pm 1038.1$ DNA molecules in one million 18S RNA, $P = 0.0004$, $P =$ NS in Combination vs. SC group) showing that further expansion of the scar could be prevented after combined cell transplantation (Fig. 5). On the other hand, Troponin T expression in the scar did not significantly differ between the groups indicating that no additional cardiomyogenesis occurred even after combined cell transplantation ($P = 0.248$).

### 3.5. Overexpression of angiogenic factors and chemoattractants in the peri-infarct area and the scar

Expression of VEGF was significantly higher both in the infarct scar (Comb: $119.3 \pm 20.9$ DNA molecules in one million 18S RNA, vs. Control: $24.1 \pm 7.5$ DNA molecules in one million 18S RNA, $P = 0.009$) and the peri-infarct area (Comb: $153.5 \pm 23.4$ DNA molecules in one million 18S RNA, vs. Control: $32.4 \pm 6.8$ DNA molecules in one million 18S RNA, $P = 0.007$) in animals after combined cell transplantation showing that both cell types possess an angiogenic potential. Combined transplantation of skeletal myoblasts and AC-133+ progenitors led to a three-fold increase in

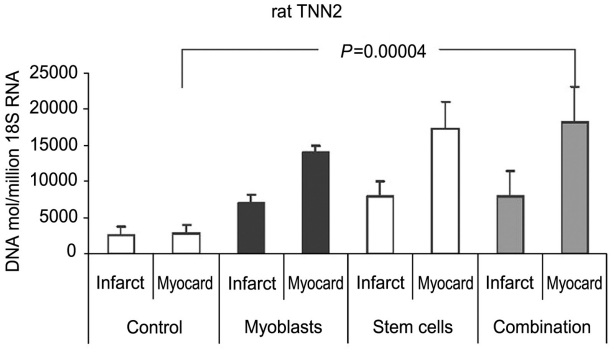


Fig. 5. Increased Troponin-T gene expression in the peri-infarct area after combined transplantation of skeletal myoblasts and AC-133+ progenitor cells detected by rt-PCR.

TGF-beta levels in the peri-infarct zone ($P=0.012$), while the increase of TGF-beta expression in the infarct area did not exceed 58%. A similar effect was detected in SDF-1 expression in the scar tissue, where an impressive almost 2.5-fold increase could be shown in the double cell therapy group as compared to controls ($P=0.00025$). As shown in Fig. 6, this increase was less prominent in the cell monotherapy groups (Comb vs. SC, $P=0.038$ and Comb vs. SM, $P=0.043$). Interestingly, no additional SDF-1 production was detected in the infarct scar, showing that the contribution of skeletal myoblasts to the secretion of this particular chemokine is limited.

## 4. Discussion

In the current study, we delved into the mechanisms of myocardial improvement after combined transplantation with skeletal myoblasts and angiopoietic progenitors in a model of ischemic heart failure. Our concept was based on a novel approach of combined cell therapy emphasizing the complementary effects of the cell types used. We therefore hypothesized that transplantation of angiopoietic progenitors in the peri-infarct area may improve cell survival and functionality of engrafted skeletal myoblasts, which in turn represent new contractile components in the infarct scar. The increased functional capability of the latter may enhance the neoangiogenic potential of transplanted AC-133 progenitors in the peri-infarct zone so that further expansion of the infarct scar can be avoided.

The major finding of this study was the increased engraftment and, most importantly, the increased survival of the transplanted cells in the combination group. The survival rates of angiopoietic progenitors in the peri-infarct area and of skeletal myoblasts in the scar were twice as high as compared with single cell therapy. Several factors such as cell isolation and preparation for injection, as well as the injection technique itself in combination with the secretion of chemoattractants from host myocardium have been associated with high engraftment rates [7–9]. Inflammation mediators such as TNF-$\alpha$, IL-8 or SDF-1 are immediately secreted and play an important role in the homing of endothelial progenitors in the mobilization of the stem cell niche after acute myocardial infarction [10]. However, taking into consideration that cytokine production and expression of chemoattractants stemming from ischemic myocardium declines within days after myocardial infarction [11, 12], cell homing can only be attributed to injection-associated local chemokine production in a setting of chronic ischemia [13].

However, even if cells have survived the implantation procedure, long-term survival is not guaranteed in this hostile environment. As shown by other investigators, the percentage of detected donor cells in the heart decreased rapidly from 34–80% of injected cells immediately after injection to 0.3–3.5% at six weeks [14]. The injected cells have been found to populate extracardiac tissues including the spleen, the liver and skeletal muscles 42 days after cell transplantation, which makes evident that not only attraction of the cells in the target area but also long-term homing is crucial for a successful cellular cardiomyoplasty. In our previous study, we showed that capillary density in the peri-infarct area was 6.5 times higher in animals after

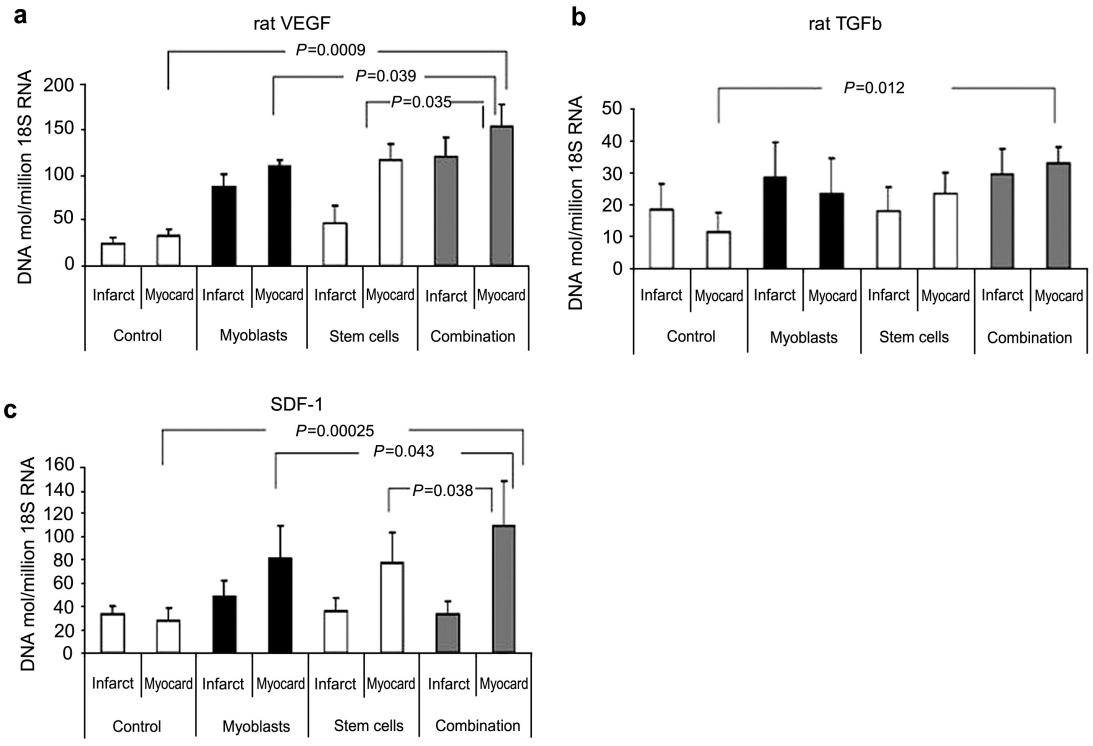


Fig. 6. Increased expression of (a) Vascular Endothelial growth Factor-A (VEGF-A), (b) Transforming Growth Factor-beta-1 (TGF-beta-1), and (c) Stromal-Derived Factor-1 (SDF-1) in the peri-infarct area and the scar after combined transplantation of skeletal myoblasts and AC-133+ progenitor cells (gray bars) compared to isolated stem cell- (open bars), skeletal myoblasts transplantation (closed bars), or controls (dotted bars) as detected by rt-PCR.

double cell therapy, which could be mainly attributed to new vessel formation stemming from the injected angiopoietic progenitors [5]. In order to better understand the mechanism of neoangiogenesis we measured the expression of the VEGF-A-gene in the heart tissue. This protein is believed to play a key role in blood vessel formation and its receptors flt-1 and flk-1 are important for endothelial differentiation, migration, proliferation and vascular remodeling [15]. We were able to show that VEGF production was almost five times higher both in the peri-infarct area and the scar, indicating that both AC-133+ progenitors and skeletal myoblasts are facilitating or even producing angiogenic factors, which in turn leads to enhanced neoangiogenesis. Our results confirm the findings of other investigators, which also demonstrated that combined cell therapy in different models of myocardial ischemia was associated with increased neoangiogenesis and higher local VEGF production as compared to single cell therapy [3]. Beside its capability to promote neovascularization, VEGF is also involved in the mobilization of stem cells from the bone marrow [16] and has a mitogenic effect on cardiomyocytes as well. In a study from Laguans et al., intramyocardial injection of VEGF resulted in a significant increase in the number of mitotic nuclei and nuclear hyperplasia, suggesting that VEGF may promote kariokenesis in cardiomyocytes [17]. In addition, upregulation of TGF-beta-1 has been associated with both cardiogenesis in vertebrate embryos [18] and induction of fibrogenesis and fibroblast stimulation by promoting cardiac angiotensin II [19].

In order to evaluate cardiomyogenesis, we measured the expression of cardiac troponin gene in infarcted myocardium. Troponin T levels were higher in the peri-infarct area but not in the infarct scar, suggesting that the effect of combined cell therapy on native cardiomyocytes was limited in avoiding expansion of the infarct scar, whereas, no evidence of transdifferentiation into new cardiomyocytes was detected. This is in alignment with our histological findings, which demonstrated a reduction of collagen deposition in the peri-infarct area, whereas the reduction of the infarct scar was only attributed to myotube formation [5]. This aspect has yielded contradicting results in the literature, as several investigators were able to demonstrate a significant myogenic potential of injected stem cells, whereas this potential has been questioned by others, which propagate that detection of new cardiomyocytes has related to fusions [20] or methodological shortcomings but not to transdifferentiation [21].

Apart from angiogenesis-related increased myoblast survival in the scar, we were able to demonstrate an increased homing and long-term survival of the injected AC-133+ cells in the peri-infarct area. This fact in combination with histological findings, suggesting that angiopoietic progenitors injected in the peri-infarct region migrate and proliferate in the infarct scar, provides evidence that increased stem cell survival and proliferation can be attributed to the presence of skeletal myoblasts, representing the second part of the interaction between the two cell types. This assumption is supported by the upregulated VEGF production in the infarct scar, where skeletal myoblasts were actually injected. Taking into consideration that secretion of angiogenic factors from other cell types in the scar is not probable, one can assume that chemoattractants are produced from engrafted myoblasts, which in turn increase homing and proliferation of the injected stem cells [22]. This hypothesis is also supported by the fact that SDF-1 production in myocardial tissue is highly upregulated in animals after combined transplantation even at eight weeks after infarction. This protein, which is physiologically over expressed after acute myocardial infarction [23], specifically interacts with the CXCR4 receptor and orchestrates the mobilization and homing of hematopoietic stem cells from bone marrow to the ischemic heart and therefore promotes angiogenesis [24]. Accordingly, Niagara et al. have been able to demonstrate increased cell survival in the infarcted myocardium by preconditioning of the transplanted skeletal myoblasts. This therapeutic strategy had a protective effect on the injected cells via release of signalling mediators and paracrine factors, which resulted in increased angiogenesis and improved cardiac function after infarction [25].

To summarize, in this study we have demonstrated that the positive effect of combined transplantation of skeletal myoblasts and angiopoietic progenitors is not an additive result of two separate cell types but the injected cells seem to act in a bidirectional synergistic way at the local level of infarcted myocardium. The mechanism of functional improvement in ischemic heart failure is mainly associated with increased angiogenesis based on paracrine factors, which results in improved survival and lower apoptosis rates of the injected cells.

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